chemical products can be avoided, however only chemical species that cannot pass through the membrane or salt bridge can be focused.

Two additional methods for concentrating a sample include sample stacking and field amplified sample injection in which a sample is concentrated as the sample crosses a boundary between low and high conductivity buffers. These methods can achieve preconcentration factors of 100 to 1000-fold although these methods require multiple buffers. Sweeping is yet another concentration method which is capable of a very high degree of sample concentration (e.g., up to 5000-fold), but is useful only for small hydrophoic analytes with a high affinity for a mobile micellular phase.

An additional technique for concentrating an ionic sample includes isoelectric focusing. Isoelectric focusing is commonly used for the concentration and separation of proteins and involves the focusing of analytes at their respective isoelectric points (pls) along a pH gradient.

Two examples of recent isoelectric focusing techniques are provided by U.S. Pat. No. 3,664,939 to Luner et al. and U.S. Pat. No. 5,759,370 to Pawliszyn. Both references relate to isoelectric focusing with pH gradients that are created by the application of a temperature gradient. The isoelectric focusing uses a pH gradient to focus analytes and in particular proteins, at their isoelectric points. The isoelectric point is the pH at which the analyte has zero electrophoretic mobility, i.e., approximately zero charge. pH gradients for isoelectric focusing are typically generated using ampholyte mixtures or immobilized ampholytes in gels. The two above referenced patents are included here as examples of prior art uses of temperature gradients for focusing. It is actually very unusual for isoelectric focusing to be done with a pH gradient generated with using a temperature gradient.

One disadvantage with isoelectric focusing is that it is limited in application because it can only be used with analytes with an accessible pl. Additionally, the concentration to which a protein can be focused with isoelectric focusing is severely limited due to the low solubility of most proteins at their pls.

BRIEF SUMMARY OF THE INVENTION

The present invention concerns a method and device for concentrating and separating ionic species in solution within fluid conduits which include channels, microchannels, and capillary tubes. The concentration is achieved by balancing the electrophoretic velocity of an analyte against the bulk flow of solution in the presence of a temperature gradient. Using an appropriate buffer, the temperature gradient can generate a corresponding gradient in the electrophoretic velocity so that the electrophoretic and bulk velocities sum to zero at a unique point and the analyte will be focused at that point. The present invention may be adapted for use with any charged analyte, including fluorescent dyes, amino acids, proteins, DNA, cells, and particles and may provide up to or, in some instances, exceed a 10000-fold concentration of a dilute analyte.

One aspect of the present invention concerns a method for directing ionic analytes contained in an ionic buffer solution of a system and which may include concentrating or separating analytes present in the buffer solution. The method includes producing an electric current flow in an ionic buffer solution containing at least one species of ionic analyte to cause the analyte ions to migrate electrophoretically. A temperature gradient is established in the buffer solution to have a significant component substantially aligned with the current flow, to thereby generating a gradient of the electrophoretic velocity of the analytes. A bulk flow is produced in the buffer solution such that the bulk flow has a significant component substantially aligned in the direction opposite the direction of the electrophoretic migration of one or more of the analytes so that the total velocity of one or more of the analytes is equal to zero at some point in the system.

According to another aspect of the present invention, a fluidic device includes a fluid conduit and an ionic buffer disposed in the conduit. At least one source or sink of heat, thermally coupled to the fluid conduit, is provided for establishing a temperature gradient having a significant component substantially aligned with the current flow so as to form an electrophoretic velocity gradient within the fluid conduit. A voltage potential source is provided for applying an electric field along a length of the fluid conduit and a current source provides an electric current flow through the ionic buffer in the fluid conduit. A source of bulk fluid flow provides for an opposing flow of the buffer in the fluid conduit. In alternate, further embodiments, the ionic buffer has either a temperature dependent ionic strength or a temperature dependent pH such that when a temperature gradient is applied to the fluid conduit, an electrophoretic velocity gradient is established in the ionic buffer present in the fluid conduit.

One advantage or feature of the present invention is provided by a technique that allows for simultaneous concentration and separation in a manner similar to isoelectric focusing but which is adoptable for use with any charged analyte and is not limited to molecules for a specific pl or range of pls. Further, the temperature gradient focusing of the present invention can be used to achieve higher degrees of sample concentration, e.g., more than 10,000 fold concentration of a dilute sample, when compared with any prior single sample preconcentration method.

A further feature of the present invention is that the electrophoretic velocity gradient is formed within the channel or capillary in response to the temperature gradient without the need for externally manipulated voltages or complicated and difficult to fabricate semi-permeable structures.

Further features and advantages of the present invention will be set forth in, or apparent from, the detailed description of preferred embodiments thereof which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in detail with respect to preferred embodiments with reference to the accompanying drawings, wherein:

FIG. **1**(*a*) is a schematic depicting a prior art microchannel device which provides for electric field gradient and FIG. **1**(*b*) is a plot of the electric field versus distance (x) along the microchannel of FIG. **1**(*a*);

FIG. **2** is a plot of velocity versus distance along the microchannel of FIG. **1**(*a*);

FIG. **3**(*a*) is a schematic illustration of temperature gradient focusing and fluid conduit in the form of a microchannel in accordance with the present invention, FIG. **3**(*b*) depicts temperature distribution along the microchannel of FIG. **3**(*a*), and FIG. **3**(*c*) is a plot of the function

$$f(T) = \frac{\sigma(20)\cdot\eta(20)}{\sigma(T)\cdot\eta(T)}$$